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Characterization of *Flavobacterium psychrophilum* isolates originating from rainbow trout farms with a high degree of water recirculation

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Abstract

The demand for increased production on Danish rainbow trout farms as well as reduced environmental impact has lead to the redesign of some of the traditional flow-through farms to model farms based on recirculation technology. Traditional farms have consisted of earth ponds and used stream water, whereas model farms are based on concrete fish tanks, biofilters and a high degree of water recirculation. Only bore-hole water is used with reduced intake (and thus water discharge) compared to traditional farms. In that way it is possible to lower the discharge of nutrients, chemicals and medicine to natural streams, and thereby making it possible to expand the fish production at a farm. At model farms there are possibilities of better controlling water parameters and diseases, resulting in improved fish health and welfare. An important factor is to use all in - all out strategies at farms for accomplishing the latter.

One of the bacterial diseases giving rise to problems at traditional freshwater farms is Rainbow Trout Fry Syndrome / Bacterial Cold Water Disease caused by *Flavobacterium psychrophilum*. The occurrence of this bacterium was investigated on the model farms, by quarterly examinations of 20 fish (of different sizes) on each of eight model farms during a 1 year period. The quarterly examinations of fish were continued on two farms for an additional year. Samples were taken from skin, gills, spleen, kidney and brain and inoculated on tryptone yeast extract salts (TYES) agar [1] for isolation of *F. psychrophilum*. Samples from skin and gills were also inoculated on TYES agar supplemented with sulfadiazin and trimethoprim, antibiotics suppressing the natural water flora but not the growth of *F. psychrophilum* [2]. The agar plates were incubated at 15°C. Positive identification of *F. psychrophilum* was done by negative growth on blood agar plates and by a species-specific PCR method [3; 4].

The bacterium was found on all eight farms, also from inner organs, but primarily from the outside of the fish. The isolates were found from fingerlings and larger fish, but not from the few fry that were investigated. The first year of sampling yielded 781 *F. psychrophilum* isolates (eight farms), whereas the following year resulted in the isolation of 186 isolates (two farms). The first year *F. psychrophilum* was isolated from 47 % of the fish (25 % of these also from inner organs), whereas the percentage had gone up to 61 the following year (33 % of these also from inner organs). The question was raised if the isolates found primarily were pathogenic or non-pathogenic, as it has been shown that *F. psychrophilum* can be divided in virulent and non-virulent strains [5].

The methods used for characterising *F. psychrophilum* have been serotyping [3], ribotyping (restriction enzyme *Eco*RI) and plasmid profiling [5], leading to experimental infection challenges by selected isolates representing different groups of isolates [6; 5] as

well as comparing with well characterised strains, among them the type strain NCIMB 1947 and the Danish strains 950106-1/1 and 900406-1/3.

Investigations done in the 1990'es on isolates originating from 5 farms (of them 2 hatcheries) showed that the 299 isolates could be divided in 7 ribotypes, where most of the isolates belonged to the ribotype A, either serotype Fd or Th (based on the serology system by [7]) and had a plasmid about 3.3 kb [5]. Experimental infection challenges showed that such isolates were virulent [5].

Other investigations done in 1999-2002 at different hatcheries showed that most of the isolates found among fry were very homogenous, most of them belonging to ribotype A, whereas isolates from broodfish were much more heterogenous. Isolates with ribotype A were also found in broodfish, in mucus of skin and gills as well as inner organs. Less virulent strains were also found both from outside as well as inner organs of the broodfish [8; 9]. Figure 1 shows some of the ribotypes found during the investigations. Experimental infection challenges have shown that isolates with ribotype A and D have been found to be virulent, whereas isolates with ribotypes B and G have been found to be less virulent [5].

Part of the isolates originating from model farms (from fingerlings and larger rainbow trout) was characterized by ribotyping. More than 20 ribotype profiles were found, among them profiles that had been found before [8; 9], but also new profiles. Some of the profiles were restricted to one farm, whereas others were found on several or all eight farms. Isolates with ribotype profiles also found among well-characterized virulent strains were found in skin, gill and ulcer samples as well as from internal organs of fish. The same pattern was seen for isolates that were expected to be less virulent judging from their ribotype profile. The most common ribotype profile found was ribotype A. Serotyping as well as plasmid profiling were done on representatives from each of the ribotype profile groups, and these preliminary results showed that ribotype A isolates were found in inner organs as well as skin/gills/ulcers and were either serotyped as Fd or Th. This is in compliance with earlier results [5; 8; 9]. Isolates that had either no plasmids or plasmids not being 3.3 kb seemed to belong to serotype Fp^T, the serotype that mostly covers less virulent isolates.

The reason for the high level of infection among fish sampled at the model farms might be that all in - all out strategies were not used on any of the farms and that new fish from traditional farms were taken in at the farms during the investigations.

In conclusion it was shown that *F. psychrophilum* isolates from fingerlings and larger rainbow trout were very heterogeneous when it came to ribotype profiles, in comparison to the more homogeneous isolates originating from fry (from disease outbreaks). Isolates with the same characterisation profiles as those from diseased fry could also be found in larger fish, both in mucus from skin and gills and ulcers as well as inner organs. Further studies are needed to see if some of the many *F. psychrophilum* isolates found are non-virulent environmental isolates. Other more descriptive molecular typing methods might be used in addition to experimental infection challenges with few selected isolates representing the different types of *F. psychrophilum*.

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Figure 1 published in Journal of Fish Diseases (Madsen & Dalsgaard 2008, 31:799-810).

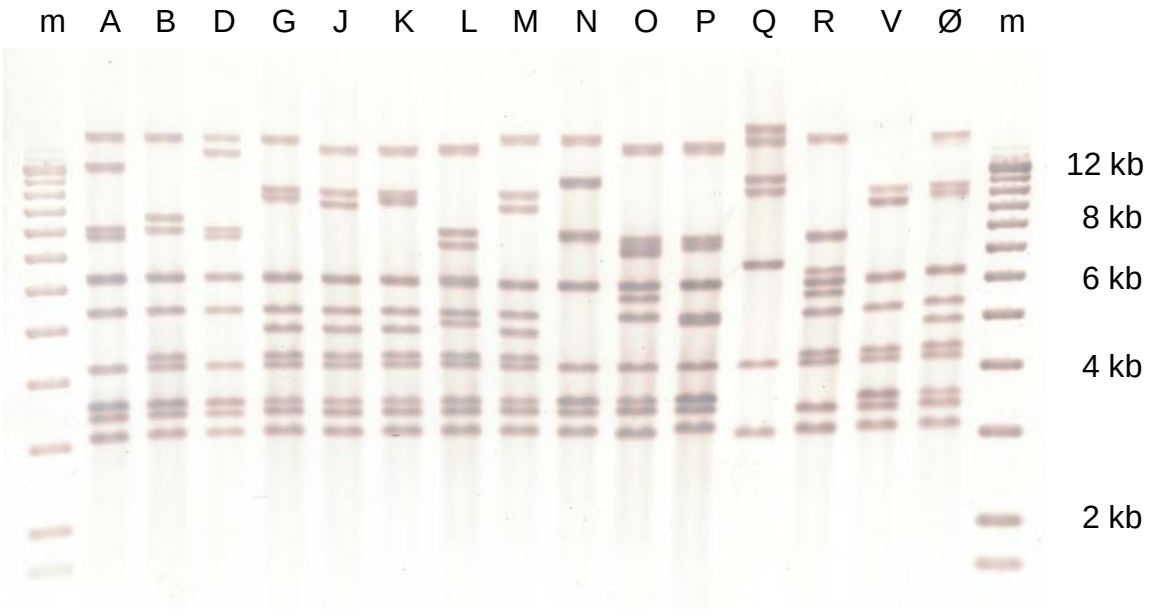

Figure 1 Ribotypes of *Flavobacterium psychrophilum isolates* obtained with EcoRI. Fifteen different ribotypes, designated A, B, D, G, J, K, L, M, N, O, P, Q, R, V and Ø are shown. Lanes m both contain a 1-kb molecular weight standard.